DEVELOPMENT OF ANION-EXCHANGE MEMBRANE CHROMATOGRAPHY FROM REGENERATED CELLULOSE MEMBRANE BY ATTACHING DIFFERENT SPACER ARM LENGTH OF DIAMINE MONOMER

YUE WEI LEE

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LIST OF ABBREVIATIONS

AA	Acrylic acid
ADP	Adenosine diphosphate
ATRP	Atom transfer radical polymerization
BSA	Bovine Serum Albumin
CA	Cellulose Acetate
DEAE	Diethyl amino ethyl
DNA	Deoxyribonucleic acid
CPES	Carboxylic polyethersulfone
DEEDA	N,N-Diethylethylenediamine
DMAEMA	poly(2-dimethylaminoethyl methacrylate)
EDGE	Ethylene glycol diglycidyl ether
EPI	Epichlorohydrin
IgG	Immunoglobulin G
IPA	Isopropanol
kDa	kilo Dalton
$KMnO_4$	Potassium permanganate
MAETMAC	(2-(Methacryloyloxy)ethyl)-trimethylammonium chloride
MF	Microfiltration
NAD	*Nicotinamide adenine dinucleotide*
NADP	*Nicotinamide adenine dinucleotide phosphate*
NF	Nanofiltration

NMP	N-methyl-2-pyrrolidone
(NTA)-CU^{2+}	Nitrilotriactetate copper
PA	Polyamide
PAN	Polyacrylonitrile
PC	Polycarbonate
PE	Polyethylene
PEGMA	Glycol methacrylate
PES	Polyethersulfone
pH	Potential hydrogen
p*I*	Isoelectric point
PI	Polyimide
Poly(MES)	poly(2-(methacryloyloxy) ethyl succinate
PP	Polyphenol
PPE	Polypropylene
PPO	Polyphenol oxidase
PVDF	Poly(vinyldene fluoride)
RC	Regenerated Cellulose
RNA	Ribonucleic acid
RO	Reverse osmosis
SPM	Sulfopropyl methacrylate
TFC	Thin film composite
UF	Ultrafiltration
UV	Ultraviolet

LIST OF SYMBOLS

J	Flux
v	Volume
A	Area
t	Time
M	Molarity (mg/ml)

PEMBANGUNAN MEMBRAN KROMATORAFI JENIS PERTUKARAN ANION DARIPADA MEMBRAN JENIS REGENERASI SELULOSA DENGAN MENLAMPIRKAN MONOMER DIAMINE YANG MEMPUNYAI PANJANG *SPACER ARM* YANG BERBEZA

ABSTRAK

Teknik kromatografi digunakan secara meluas untuk pemisahan protein. Kromatografi turus terpadat yang biasa digunakan mempunyai beberapa kekurangan. Membran kromatografi adalah alternative teknik yang sesuai untuk pemisahan protein. Monomer tertentu boleh dilekatkan ke atas membran yang tidak bercas untuk mengubahsuainya kepada membran kromatografi. Pengoptimuman parameter-parameter yang terlibat dalam modifikasi secara kimia ini adalah penting untuk menghasilkan membran kromatografi yang berprestasi tinggi dalam aplikasi pemisahan protein. Tujuan kajian ini adalah untuk menghasilkan membran chromatography jenis pertukaran anion daripada membran jenis regenerasi selulosa dengan melekatkan monomer diamine monomer yang mempunyai panjang *spacer arm* yang berbeza. Membran regenerasi selulosa diaktifkan dalam larutan yang mengandungi natrium hidroksida (NaOH) dan epichlorohydrin (EPI). Kemudian, membran itu direndam dalam larutan diamine 1,2-diaminoethane atau 1,4-diaminobutane bagi menghasilkan membrane kromatografi bercas positif. Kepekatan NaOH semasa pengaktifan dari 0.05M ke 0.50M dan kepekatan monomer diamine dari 0.25M ke 2.0M semasa *grafting* telah dikaji. Kepekatan NaOH yang optima ialah 0.20M yang menghasilkan membran kromatografi jenis pertukaran anion berkapasiti 0.310 ± 0.033 mgBSA/cm^2 membran. Kepekatan monomer diamine yang tinggi pada 2.0M 1,4-diaminobutane memberikan membran berkapasiti 0.385 ± 0.027mgBSA/cm^2 membran. Dengan merujuk kepada puncak transmisi graf FTIR, kedua-dua kumpulan berfungsi N-H dan C-N wujud dalam membran yang diubahsuai mengambarkan kejayaan process *grafting*.

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ABSTRACT

Chromatography technique is widely used for protein separation. Conventional packed bed column chromatography has several limitations. Membrane chromatography was a suitable alternative technique for protein separation. Specific monomer can be grafted to uncharged membrane to transform into membrane chromatography material. Optimization of parameters involve during this chemical modification is crucial for the development of high performance membrane chromatography for protein separation. The purpose of this research is to develop anion-exchange membrane chromatography from regenerated cellulose membrane by attaching different spacer arm lengths of diamine monomer. Regenerated cellulose membrane was activated in a solution containing sodium hydroxide (NaOH) and epichlorohydrin (EPI). Then, the membrane was immersed in diamine solution of 1,2-diaminoethane or 1,4-diaminobutanhe to produce positively charged membrane chromatography. The concentration of NaOH activation from 0.05M to 0.50M and diamine monomer concentration from 0.25M to 2.0M during grafting were studied. The optimum concentration of NaOH was 0.20M which produced anion exchange membrane capacity of 0.310 ± 0.033 mgBSA/cm^2 membrane. High concentration of diamine monomer at 2.0M 1,4-diaminobutane showed a binding capacity of 0.385 ± 0.027mgBSA/cm^2 membrane. Based on FTIR transmission peak, both N-H and C-N functional groups were detected in modified membrane that indicated the successful of grafting process.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Conventional techniques for separation involve several steps such as impurities removal, isolation, purification and polishing. More than 60% of the total cost of bioprocess in worldwide was due to downstream recovery and purification. High efficiency and high productivity separation techniques were essential to pharmaceutical industry. Besides that, increasing industrial demand of food products for large-scale extraction and purification had cause the separation process to further develop and exploit. In food sector, more advance separation processes have been developed for concentration and fractionation of molecules from raw material such as separation of protein from milk. Many biological active components have potential in nutraceutical applications and the global functional food market was expected to growth approximately 8% per year. At this rate, the market will be valued more than US$100 billion in year 2012 (Smither, 2008).

Chromatography technique was widely used for protein separation in the packed bed configuration. However, conventional packed bed column

chromatography have several limitations such as high pressure drop, long processing times due to slow pore diffusion and complicated scale up procedures (Ghosh, 2003). Sometimes, channelling can occurred due to cracking of packed bed which caused a major problem. Membrane chromatography is becoming more popular as alternative to the packed bed chromatography. It is a combination of chromatographic principle and membrane filtration methods. Membrane chromatography shows several advantages such as low pressure drop, fast protein accessible to the specific functionality in the membrane by bulk convection with only little pore diffusion, easy to scale up and set up.

Highly adsorptive membranes can be prepared in three general steps involving preparation of base membrane, chemical activation of the base membrane and coupling of ligands or specific functional group to the activated membrane (Zheng & Ruckenstein, 1998). Membrane with good performance should include excellent mechanical strength, great oxidative, thermal and hydrolytic stability as well as good-forming properties (Zou et al., 2001).

Various methods used to prepare charged membrane chromatography. Optimization of both preparation method and the chromatography process are necessary in order to obtain higher performance membrane chromatography process. Protein transport by membrane was affected by electrostatic interaction between charged protein and charged membrane. The membrane surface varies according to the type of ligand coupled on it. Molecular structure of charged ligand has impact on the membrane adsorption characteristics. In this study, commercial regenerated

cellulose microfiltration membrane will be modified into anion-exchange by attachment of amine based functional group to create affinity toward anionic protein.

1.2 Problems Statement

Chromatography in a packed bed configuration is commonly used technique for protein separation and isolation. However, several limitation of packed bed column chromatography have been identified such as high pressure drop, long processing times with slow diffusion and complicated scale up procedures (Ghosh, 2002; Kawai et al, 2003). In contrast with column chromatography, membrane chromatography has advantage such as lower pressure drop, higher productivity and easy scale up. Unwanted fouling or clogging is minimized in membrane chromatography and give a promising large-scale production for separation and recovery of protein.

Anion-exchange membrane chromatography with high binding capacity has potential for commercial application in industries. Anion-exchange membrane can be prepared by chemical grafting, UV-grafting, photo-grafting, polymer grafting and etc (Bhattarcharya & Misra, 2004). Negatively charged protein can be separate selectively and effective by using positively charged membrane. Suitable ligand need to be selected to modify existing membrane with positive functional group for adsorption on selective anion charged protein. The parameters involve in synthesis route to produce anion-exchange need to be further study, analyze and characterize to produce high performance membrane chromatography.

Spacer arm lengths of diamine which use as positive charged functional ligand on membrane surface have strong effect on the protein binding capacity. Different spacer arm length, determine by the number of alkyl groups between membrane and functional ligand, having different protein binding and behaviour. The relationship of spacer arm length as one of the ligand properties is essential to study in order to develop membrane with higher performance applications.

1.3 Research Objective

The main objective of this research was to study the effects of parameters involve in preparing anion-exchange membrane chromatography from regenerated cellulose membrane using different spacer arm length of diamine monomer.

1.4 Research Scopes

With the intention of fulfil the objective of the research; the following scopes have been outlined:

i. Study the performance of anion-exchange membrane chromatography with different spacer arm length of diamine monomer which are 1,2-diaminoethane and 1,4-diaminobutane.
ii. Study the effect of NaOH concentration from 0.05M to 0.50M during the activation of regenerated cellulose membrane.
iii. Study the effect of diamine monomer concentration from 0.25M to 2.0M on the protein binding capacity.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Proteins are essential to all living organisms for regulation, function and structure of the body. Development of protein begins with DNA transcribing into RNA and finally RNA translating into proteins. Protein comprised of polymer chains where amino acids linked together by a strong peptide bonds (Hagel et al., 2008). There are more than twenty different types of amino acid use to form protein and each distinguished by functional "R" group. When two amino acids linked together is called a dipeptide and many linked together called as polypeptide. Polypeptide consists of a backbone and side chains. The backbone comprises of amide nitrogen, α- carbon and carbonyl carbon. Some examples of proteins are hormones, enzyme and antibodies. The side chains comprise the functional "R" group. The basic structure of amino acid was shown in Figure 2.1.

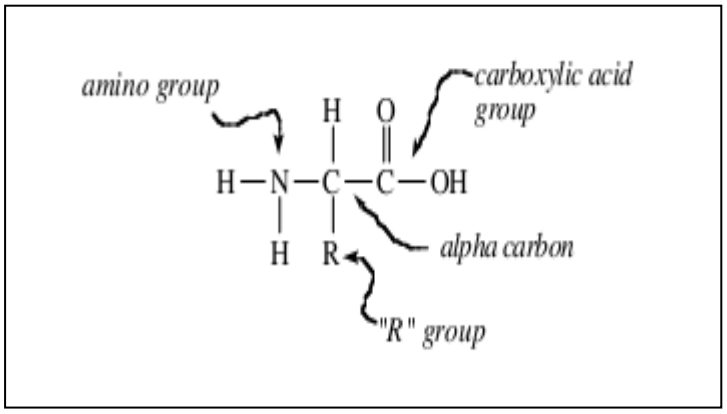

Figure 2.1 Basic structure of amino acid.

Protein is major nutrition needed by human and other living organism. It involves in building body cells and enhances growth, forming blood and maintenance of human body. When protein break down into amino acids, these amino acids functions as major abundant molecule for body parts and act as a precursors to hormones, immune response, cells and molecules repair and co-enzymes (Gregory et al., 2004).

Protein is becoming important in biopharmaceutical and food processing industries. Production of proteins especially for human use is essential to replace the extraction of proteins directly from natural sources to minimize the risk of poisonous impurities enters into human body. Protein-based drugs were growing in ultimate speed for treatment of various diseases in humans and animals. Various single proteins were use in wide range of applications. Bovine serum albumin (BSA) as an example has numerous applications such as act as nutrient in cell and microbial culture. BSA stabilizes some enzymes during DNA suggestion and involve in quantitative determination of other proteins. Immunoglobulin act as an antibody and antigen in medical application especially known as Rho(D) immune globulin

antibodies which against Rhesus factor. Wide applications of protein proven that single protein have huge potential in economic and social development (Petsko & Ringe, 2004).

2.1.1 Properties of Protein

A protein will have a positive net charge at low pH and negative net charge at high pH. This indicate that pH have large influence on the net charge of a protein. This is due to the charged of amino acids and also dissociated carboxylic acid group of a protein. At particular pH where the surface carries no net electric charge is called as isoelectric point (p*I*). Proteins isoelectric point mostly dependes on seven charged amino acids which are glumate (*δ-carboxyl group*), aspartate (β-carboxyl group), cysteine (thiol group), tyrosine (phenol group), histidine (imidazole group), lysine (ε-ammonium group) and arginine (guanidinium group). Positive charges are usually provided by arginie, lysine and histidine, depending on surrounding buffer pH. The protein terminal group (NH_2-COOH) should take into account charge as each of them has its unique acid dissociation constant referred as pK. The net charge of protein is related to the pH of buffer solution. Handerson-Hasselback equation can be used to calculate protein charge in certain pH. The equation is useful for estimating a buffer solution (Henry et al., 2001). Protein can be retarded by cation exchanger above the pI and by anion exchanger below the pI. Proteins normally show absorption at 280nm due to the peptide bond (Hagel et al., 2008).

2.1.2 Bovine Serum Albumin

BSA had a number of amino acid about 583 with molecular weight of 66.5kDa and isoelectric point (p*I*) of 4.7 at 25℃. Isolated BSA was found to be a very functional protein. BSA involves in numerous biochemical applications such as immunoblots, immonuhistochemistry and enzyme linked immunosorbent assay. BSA also functions as a nutrient in cell and microbial culture. BSA acts as a base for preparation of defined fatty acid supplements and help enzyme stabilization during purification stage. BSA prevents adhesion of enzymes to reaction tips and tube surfaces and also acts as a blocking agent to minimize background in protein and deoxyribonucleic acid (*DNA*) labelling (Acton, 2011).

2.2 Methods for Protein Separation

Protein separation can be divided into two different methods that are analytical and preparative scale. Preparative method is more suitable for large scale production in industry. Extraction is one of the techniques that commonly use to break the cells or tissue to obtain the proteins of interest. A few method need to undergo throughout extraction process such as freezing, sonication and filtration. The method depends on fragility of the protein. Soluble protein will be in the solvent after extraction and can be separate from cell membranes by centrifugation.

Precipitation is a common method use to isolate bulk protein with use of ammonium sulfate $(NH_4)_2SO_4$. Different fractions of precipitate protein can be collect by increasing amounts of ammonium sulfate. The process based on some

properties of the solvent such as addition of salts, organic solvents and polymer or by manipulating pH or temperature. Dialysis will be carrying out later to remove ammonium sulfate. Protein will get aggregated when hydrophobic protein groups attracts toward other hydrophobic groups. This method suitable to be use for large volume isolation due to lower operating cost (Deutscher, 1990). Precipitation is the only practical way to separate different types of protein in early days.

Ultracentrifugation is another alternative method for protein separation by using centrifugal force to separate protein mixture. The mixture usually contains varying types and densities of suspended particles suspended inside it. When a vessel containing proteins rotate at high speed for a constant time, a momentum yields an outward force to each particle that is proportional to its mass.

Chromatographic methods widely used in industries where the separation of protein can be done more efficiency and in larger scale. Different protein will interact differently with column material and elution process was required to carry out to recovered bound protein (Charcosset, 1998). There are different chromatographic modes available such as size exclusion chromatography, ion exchange chromatography and affinity chromatography. Chromatography based method has high resolving power and become dominant for protein separation (Hedhammar et al., 2011).

2.3 Chromatographic Methods

Chromatographic methods for proteins separation have been developed for commercial scale production. Chromatography refers to separation techniques that involve retardation of molecules with respect to the solvent front that movement through the material. It refers to resolution of solutes by differential migration during passage through a porous medium. In chromatographic techniques, the separation principle operates in different migration of component between stationary phase and mobile phase. Another component of chromatographic system is inert support or matrix.

The separation of one protein from one to another is mainly base on properties of proteins which different from non-protein contaminants. These properties are size, charge, hydrophobicity and specific biological interaction. Several type of liquid chromatography differ mainly on the types of stationary phase involve in separation of protein. There are various types of interactions available such as ion exchange, hydrophobic, reverse phase and size exclusion chromatography. The different among these interactions is on the mechanism of separation and stationary and mobile phase used to perform the separation. For example, size exclusion chromatography is based on protein size for fractionation and ion exchange chromatography depends on charge of protein (Hedhammar et al., 2011) as shown in Figure 2.2.

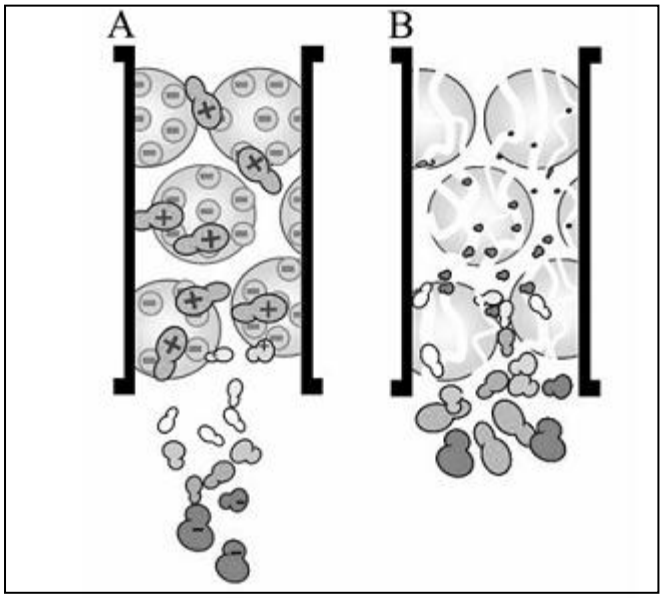

Figure 2.2 Illustrations of (A) ion exchange chromatography and (B) size exclusion chromatography (Source: Hedhammar et al., 2011)

The stationary phase known as adsorbent can either be solid or liquid. A liquid stationary phase will be held stationary by a solid support or matrix. Mobile phase which also known as solvent are normally in gaseous or liquid phase. The advantages and disadvantages for different types of stationary matrix in chromatography process as summarized by Ghosh (2003) are showed in Table 2.1.